for the specificity of the *Drosophila* proteins could be interactions with other chromatin components that are also enriched in RED chromatin.

Follow-up experiments will improve our understanding of these intriguing new colors of chromatin and their interplay with DNA-binding factors. Combined with data on other epigenomic variables such as replication initiation (Gilbert, 2001), repair (Groth et al., 2007), nucleosomal turnover (Henikoff, 2008), and three-dimensional genome organization (Cockell and Gasser, 1999), these results will lead to a more comprehensive picture of chromatin architecture and function. Clearly, it is time to say goodbye to the black and white world of heterochromatin and euchromatin.

REFERENCES

Cockell, M., and Gasser, S.M. (1999). Curr. Opin. Genet. Dev. *9*, 199–205.

Eissenberg, J.C., James, T.C., Foster-Hartnett, D.M., Hartnett, T., Ngan, V., and Elgin, S.C. (1990). Proc. Natl. Acad. Sci. USA *87*, 9923–9927.

Felsenfeld, G., and Groudine, M. (2003). Nature *421*, 448–453.

Filion, G.J., van Bemmel, J.G., Braunschweig, U., Talhout, W., Kind, J., Ward, L.D., Brugman, W., de Castro, I.J., Kerkhoven, R.M., Bussemaker, H.J., et al. (2010). Cell *143*, this issue, 212–224.

Gilbert, D.M. (2001). Science *294*, 96–100.

Groth, A., Rocha, W., Verreault, A., and Almouzni, G. (2007). Cell *128*, 721–733.

Heitz, E. (1928). Jahrb Wiss Botanik *69*, 762–818.

Henikoff, S. (2008). Nat. Rev. Genet. *9*, 15–26.

van Steensel, B., Delrow, J., and Henikoff, S. (2001). Nat. Genet. *27*, 304–308.

The Myc Connection: ES Cells and Cancer

Michael E. Rothenberg,[1,4] Michael F. Clarke,[2,4] and Maximilian Diehn[3,4,*]
[1]Division of Gastroenterology and Hepatology, Department of Medicine
[2]Division of Oncology, Department of Medicine
[3]Department of Radiation Oncology
[4]Cancer Center and Institute for Stem Cell Biology and Regenerative Medicine
Stanford University School of Medicine, Palo Alto, CA 94305, USA
*Correspondence: diehn@stanford.edu
DOI 10.1016/j.cell.2010.09.046

Gene profiling experiments have revealed similarities between cancer and embryonic stem (ES) cells. Kim et al. (2010) dissect the gene expression signature of ES cells into three functional modules and find that the Myc module, including genes targeted by Myc-interacting proteins, accounts for most of the similarity between ES and cancer cells.

Modern techniques in stem cell biology in the postgenomic era have led to dramatic advances in our understanding of the molecular underpinnings of both embryonic stem (ES) cells and cancer. Several essential "core" pluripotency genes regulating the ES cell fate (including Oct4, Sox2, and Nanog) have been defined in both mice and humans, and biologists are now using gene expression profiling experiments to discover genome-wide "signatures" for ES and cancer cells. Intriguing similarities between ES cells and cancer have arisen in such experiments, suggesting that cancers and ES cells may share fundamental mechanisms for self-renewal and differentiation (Ben-Porath et al., 2008; Somervaille et al., 2009; Wong et al., 2008). On the other hand, the similarity in gene expression between some cancers and ES cells has been puzzling because a core "stemness" signature that is shared between ES cells and other tissue stem cells has remained elusive (Fortunel et al., 2003). In addition, most human tumors do not exhibit true pluripotency. So, how can we explain the similarities in gene expression patterns between ES and cancer cells?

In this issue of *Cell*, Kim et al. address this question by carefully scrutinizing the ES cell signature and breaking it down into several functional units. Using this approach, the authors show that the connections between ES cells and cancer are largely due to Myc, the well-studied proto-oncogene that regulates many aspects of gene expression, proliferation, and differentiation in adult tissues (Kim et al., 2010).

Using a powerful, highly stringent, and innovative in vivo biotinylation technique to probe protein-protein and protein-DNA interactions (Kim et al., 2009), the authors begin by defining a Myc-centered protein interaction network in mouse ES cells. They show that this Myc complex likely interacts with the NuA4 histone acetyltransferase (HAT) complex, a highly conserved protein complex involved in diverse functions, including histone acetylation. This suggests an important role for Myc in epigenetic regulation in ES cells. The authors then use chromatin immunoprecipitation (ChIP) to define the transcriptional targets of this Myc complex. Myc targets with the most

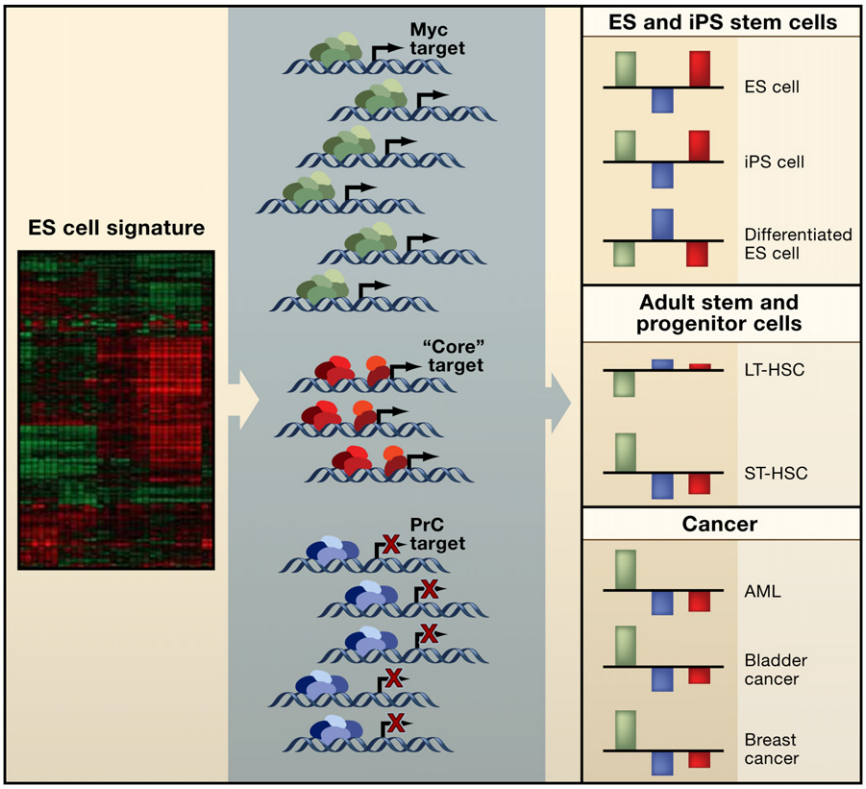

Figure 1. Components of the ES Cell Signature

Kim et al. (2010) analyze the regulatory regions of target genes for transcription factor co-occupancy. By analyzing chromatin immunoprecipitation (ChIP) data from their own experiments on the Myc protein complex in embryonic stem (ES) cells and other published ChIP experiments on different transcription factors in ES cells, they separate the ES cell transcriptional signature (far left) into three distinct modules (indicated in the gray box): the Myc module (green), the Polycomb module (blue), and the Core module (red). The authors then analyze the expression levels of these modules in various scenarios. High expression of the Myc module (green bars) is a shared property of ES cells, induced pluripotent stem (iPS) cells, short-term hematopoietic stem cells (ST-HSCs), and various cancers. However, long-term hematopoietic stem cells (LT-HSCs) and differentiated ES cells exhibit low Myc module expression. Of note, the Core (red) module—those genes targeted by Oct4, Sox2, and Nanog—is only predominant in ES and iPS cells.

Myc-associated factors bound to their regulatory regions are positively associated with epigenetic marks of active chromatin—H3 and H4 histone acetylation and H3K4 trimethylation—consistent with their data suggesting a connection between Myc and epigenetic regulation.

Kim et al. then use this Myc complex ChIP data set and other previously published ChIP experiments to obtain a more complete characterization of the targets of important transcription factors in ES cells. From there, they define three separate target gene modules based on factor co-occupancy in the regulatory regions of those target genes (Figure 1). Together, these modules constitute the ES gene expression signature: a Polycomb cluster (genes bound by the Polycomb complex factors), a Core cluster (genes targeted by the core pluripotency factors Oct4, Sox2, and Nanog), and a Myc cluster (genes targeted by the Myc-interacting proteins). These modules appear to be functionally significant, as they behave independently in different scenarios, such as during ES cell differentiation. Although previous studies had suggested that the Myc pathway is a major component of the link between ES cells and some cancers (Wong et al., 2008), it remained unclear whether Myc activates fundamental core ES cell programs such as pluripotency and self-renewal in both contexts or whether the Myc pathway is coincidentally utilized for other reasons by both ES cells and some cancers. The current study by Kim et al. clarifies this point and suggests the latter to be the case.

After defining these three separate gene expression submodules, the authors analyze gene expression data from several different cancers in both mice and humans to obtain a more precise understanding of how the ES cell signature relates to gene expression changes in cancer. This analysis shows that the Myc module is highly expressed and dominant in multiple scenarios: Myc-transformed human epithelial cancers, several mouse myeloid leukemias, some human bladder cancers, and some human breast cancers. Of interest, the Core ES cell module is not significantly expressed in these situations. Thus, in the end, Myc–rather than the core pluripotency factors or the Polycomb proteins—seems to be the common thread that ties ES cells to cancer. But what is Myc's precise role in ES cells and these cancers, particularly as it relates to self-renewal, a hallmark of stem cells? Is it inhibiting differentiation (Prochownik and Kukowska, 1986), regulating apoptosis, controlling proliferation, or performing some other function or some combination of functions?

Although Myc may affect self-renewal capacity in ES cells and cancer, it may not be a central player in this process. For example, although Myc can increase the efficiency of the generation of induced pluripotent stem (iPS) cells, it is not strictly required for reprogramming (Jaenisch and Young, 2008). In agreement with this finding, Kim et al. convincingly demonstrate that the Myc module is independent of the core pluripotency module in ES and iPS cells. Similarly, they show that, in the normal mouse hematopoietic system, the Myc module appears to segregate away from the property of long-term self-renewal. Specifically, the Myc module is upregulated in highly proliferative short-term hematopoietic stem cells (bearing the marker profile $Lin^{-}cKit^{+}Sca1^{+}CD34^{+}$)—which are more akin to progenitors, given that they lack sustained self-renewal—and not in the mostly quiescent long-term self-renewing hematopoietic stem cells ($Lin^{-}cKit^{+}Sca1^{+}CD34^{-}$), which do not exhibit Myc module expression. Thus, in the hematopoietic lineage, the proliferating progenitor is actually the cell that upregulates Myc targets rather than the self-renewing stem cell. This suggests that the presence

of the Myc module in gene expression signatures from ES cell populations and poor prognosis cancers may be more of a reflection of the active proliferation occurring in both rather than self-renewal.

In cancer, Myc's relationship to self-renewal is complex (Arvanitis and Felsher, 2006), and sometimes Myc expression may correlate with self-renewal. For example, the authors show that, in various mouse models of acute myelogenous leukemia (AML), the activity of the Myc module trends with the frequency of self-renewing leukemia-initiating cells. Although these findings could potentially be explained by the closer resemblance of the leukemia-initiating cells in AML to progenitors rather than hematopoietic stem cells (Majeti et al., 2007), further work on Myc is needed to decipher its precise role(s) in the regulation of proliferation, apoptosis, or differentiation in various stem cell settings, including ES cells, iPS cells, adult stem cells, and cancer cells.

Ultimately, by focusing on factor co-occupancy of target genes in ES cells and thereby taking a modular look at gene expression in ES cells and cancer, this paper helps us to understand the basis for their similarities. It illustrates the important role of Myc and will likely spur cancer biologists to further clarify the precise role of Myc in tumor biology, a question with potential therapeutic ramifications (Arvanitis and Felsher, 2006).

REFERENCES

Arvanitis, C., and Felsher, D.W. (2006). Semin. Cancer Biol. *16*, 313–317.

Ben-Porath, I., Thomson, M.W., Carey, V.J., Ge, R., Bell, G.W., Regev, A., and Weinberg, R.A. (2008). Nat. Genet. *40*, 499–507.

Fortunel, N.O., Otu, H.H., Ng, H.H., Chen, J., Mu, X., Chevassut, T., Li, X., Joseph, M., Bailey, C., Hatzfeld, J.A., et al. (2003). Science *302*, 393.

Jaenisch, R., and Young, R. (2008). Cell *132*, 567–582.

Kim, J., Cantor, A.B., Orkin, S.H., and Wang, J. (2009). Nat. Protoc. *4*, 506–517.

Kim, J., Woo, A.J., Chu, J., Snow, J.W., Fujiwara, Y., Kim, C.G., Cantor, A.B., and Orkin, S.H. (2010). Cell *143*, this issue, 313–324.

Majeti, R., Park, C.Y., and Weissman, I.L. (2007). Cell Stem Cell *1*, 635–645.

Prochownik, E.V., and Kukowska, J. (1986). Nature *322*, 848–850.

Somervaille, T.C., Matheny, C.J., Spencer, G.J., Iwasaki, M., Rinn, J.L., Witten, D.M., Chang, H.Y., Shurtleff, S.A., Downing, J.R., and Cleary, M.L. (2009). Cell Stem Cell *4*, 129–140.

Wong, D.J., Liu, H., Ridky, T.W., Cassarino, D., Segal, E., and Chang, H.Y. (2008). Cell Stem Cell *2*, 333–344.